

Remarks/Arguments

Reconsideration of the above-identified application in view of the present amendment is respectfully requested. By the present amendment, claims 1, 6-7, 16, 19, 21, 27, and 29-30 have been amended, claim 5 has been cancelled, and claim 31 has been added.

Below is a discussion of the 35 U.S.C. §103 rejection of claim 1, the 35 U.S.C. §103 rejection of claims 1-4 and 15, the 35 U.S.C. §103 rejection of claims 16, 22, 24-25 and 27, the 35 U.S.C. §103 rejection of claims 17-19 and 21, the 35 U.S.C. §103 rejection of claims 7-9 and 13-14, the 35 U.S.C. §103 rejection of claims 16, 23, 25-27 and 29, the 35 U.S.C. §102(e) rejection of claim 30, the 35 U.S.C. §112, second paragraph, rejection of claims 21, 27 and 30, the objections to claims 1 and 7, and the patentability of claim 31.

1. 35 U.S.C. §103(a) rejection of claim 1.

Claim 1 was rejected under 35 U.S.C. §103(a) as being unpatentable over Danek *et al.* ("Voltammetric Studies on Kinetics of Uptake and Efflux at Catecholamine Transporters," *Methods in Enzymology*, vol. 296, pp. 649-660) (hereinafter, "the Danek reference") in view of Earles *et al.* ("Rotating Disk Electrode Voltammetric Measurements of Dopamine Transporter Activity: An Analytical Evaluation," *Analytical Biochemistry* 264, 191-198 (1998)) (hereinafter, "the Earles reference"). The Office Action argues that the Danek reference discloses a method of measuring efflux of a chemical from a cell or a population of cells by introducing the chemical to the cell and measuring an electrochemical property of a medium surrounding the cell with an electrochemical system which includes a working

electrode and a reference electrode, the property being related to a concentration of the chemical medium, the method characterized by adding oxygen to the medium. The Office Action further argues that although the Danek reference does not mention that the oxygen is to increase the signal strength of the electrochemical property, this is inherent because the 95% O₂/5% CO₂ stream is provided to maintain the cells in a viable state and, if oxygen was not provided, that some cells would likely die or deteriorate and thus lower the signal strength.

As amended, claim 1 recites a method of measuring efflux of a chemical from a cell, or a population of cells, the method including introducing the chemical to the cell and measuring an electrochemical property of a medium surrounding the cell with an electrochemical system which includes a working electrode and a reference electrode, the property being related to a concentration of the chemical in the medium, the method characterized by adding oxygen to the medium to increase a signal strength of the electrochemical property, the step of adding oxygen to the medium including bubbling an oxygen-containing gas into the medium. Support for amended claim 1 can be found on page 38, lines 30-31, of the present Application.

It is respectfully submitted that the Danek and Earles references do not teach or disclose the method of amended claim 1. The Office Action states that the Danek reference as modified by the Earles reference teaches away from the feature requiring "the step of adding oxygen to the medium including bubbling an oxygen-containing gas into the medium" because "[b]ubbling the 95% O₂ – 5% CO₂ directly into the solution during an experiment is not recommended because it is likely to

result in bubbles on the electrode surface which interfere with the detection of the compound under study.”

Because amended claim 1 includes the additional feature that “the step of adding oxygen to the medium including bubbling an oxygen-containing gas into the medium,” it is respectfully submitted that amended claim 1 is not obvious over the Danek reference in view of the Earles reference. Accordingly, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claim 1 be withdrawn.

2. 35 U.S.C. §103(a) rejection of claims 1-4 and 15.

Claims 1-4 and 5 were rejected under 35 U.S.C. §103(a) as being unpatentable over Yi *et al.* (“Continuous in Situ Electrochemical Monitoring of Doxorubicin Efflux from Sensitive and Drug-Resistant Cancer Cells,” *Biophysical Journal*, vol. 75, Nov. 1998, 2255-2261) (hereinafter, “the Yi reference”) in view of the Earles reference and the CAPLAS abstract (“Growth behavior of Chinese hamster ovary cells in a compact loop bioreactor: 1. Effects of physical and chemical environments,” *Journal of Biotechnology* (1990), 15, 101-11) (hereinafter, “the Kurano reference”).

The Office Action argues that the Yi reference discloses a method of measuring efflux of a chemical from a cell or population of cells, the method including introducing the chemical to the cell and measuring an electrochemical property of a medium surrounding the cell with an electrochemical system which includes a working electrode and a reference electrode, the property being related to a concentration of the chemical in the medium. The Office Action further argues that the Yi reference does not mention adding oxygen to the medium to increase signal

strength of the electrochemical property, but that the Earles reference teaches adding oxygen to the medium. Therefore, the Office Action argues, it would have been obvious to one with ordinary skill in the art at the time of the invention to add oxygen to the medium because the oxygen would keep the cells viable and serve to increase signal strength.

As discussed above, claim 1 was amended to include the feature that “the step of adding oxygen to the medium including bubbling an oxygen-containing gas into the medium.” As also discussed above, the Office Action states that the Danek reference as modified by the Earles reference teaches away from the feature requiring “the step of adding oxygen to the medium including bubbling an oxygen-containing gas into the medium” because “[b]ubbling the 95% O₂ – 5% CO₂ directly into the solution during an experiment is not recommended because it is likely to result in bubbles on the electrode surface which interfere with the detection of the compound under study.” Because amended claim 1 includes this additional feature, it is respectfully submitted that amended claim 1 is not obvious over the Yi reference in view of the Earles reference.

Accordingly, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claim 1 be withdrawn. Because claims 3-4 and 15 depend directly from amended claim 1, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claims 3-4 and 15 also be withdrawn.

3. 35 U.S.C. §103(a) rejection of claims 16, 22, 24-25 and 27.

Claims 16, 22, 24-25, and 27 were rejected under 35 U.S.C. §103(a) as being unpatentable over the Yi reference in view of Belmont *et al.* (U.S. Patent No.

6,900,043 B1) (hereinafter, "the Belmont reference"). The Office Action argues that the Yi reference discloses an apparatus for measuring the efflux of a chemical from a biological cell, or a population of cells, including a substrate having a surface which receives the cell, a medium on the substrate, and an electrochemical monitoring system which measures an electrochemical property of the medium surrounding the cell, the property being related to a concentration of the chemical in the medium. The Office Action also argues that the Belmont reference teaches encircling cells on a substrate with a hydrophobic pen and, thus, that it would have been obvious to one with ordinary skill in the art at the time of the invention to create a hydrophobic region on the substrate which resists attachment of cells and prevents the cells from falling or moving off of the slide.

As amended, claim 16 recites an apparatus for measuring efflux of a chemical from a biological cell, or a population of cells, the apparatus including: a substrate having a surface which receives the cell; a medium on the substrate; and an electrochemical monitoring system which measures an electrochemical property of the medium surrounding the cell, the property being related to a concentration of the chemical in the medium. The apparatus is characterized by: the substrate surface having at least one attachment region to which the cell or population of cells attaches, the region being surrounded by a resistant region which resists the attachment of cells, the electrochemical system including a carbon electrode which extends at an angle relative to the at least one attachment region for measuring the electrochemical property of the medium surrounding the cell. Support for amended claim 16 can be found in Figure 1 of the present Application.

It is respectfully submitted that the Yi and Belmont references do not teach or disclose the apparatus of amended claim 16. The Yi reference teaches an electrochemical monitoring system having a carbon fiber electrode positioned horizontally relative to the substrate which receives the cell or population of cells (Figure 1). Conversely, the electrochemical monitoring system illustrated in Figure 1 of the present Application includes a carbon fiber electrode extending at an angle relative to the at least one attachment region. Because the Yi reference does not disclose an electrochemical monitoring system having a carbon fiber electrode extending at an angle to the substrate, Applicants respectfully submit that amended claim 16 is not obvious in view of the Yi and Belmont references. Accordingly, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claim 16 be withdrawn. Because claims 22 and 24-25 depend directly from claim 16, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claims 22 and 24-25 also be withdrawn.

As amended, claim 27 recites a method of measuring efflux of a chemical from a biological cell, or a population of cells, the method including introducing the chemical to the cell and measuring an electrochemical property of a medium surrounding the cell or population of cells, the property being related to a concentration of the chemical in the medium. The method is characterized by: positioning the cell or population of cells on a surface of a substrate by attachment of the cell or the cell population to an attractive region of the substrate which permits attachment, the attractive region of the substrate being surrounded by a region which resists attachment of cells, the electrochemical system including a carbon electrode

which extends at an angle relative to the at least one attachment region for measuring the electrochemical property of the medium surrounding the cell. Support for amended claim 27 can be found at page 32, line 30, to page 42, line 24 of the present Application.

It is respectfully submitted that the Yi and Belmont references do not teach or disclose the method of amended claim 27. One feature of amended claim 27 recites that the electrochemical system include a carbon electrode which extends at an angle relative to the at least one attachment region for measuring the electrochemical property of the medium surrounding the cell. As stated in the Office Action, the working electrode disclosed in the Yi reference is "a linear carbon fiber 'placed horizontally just on top of the monolayer of the cells.'" Because claim 27 includes the feature that the working electrode include a carbon electrode extending *at an angle* relative to the attachment region, and not a horizontally placed carbon electrode, Applicants respectfully submit that amended claim 27 is not obvious over the Yi and Belmont references.

Accordingly, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claim 27 be withdrawn.

4. 35 U.S.C. §103(a) rejection of claims 17-19 and 21.

Claims 17-19 and 21 were rejected under 35 U.S.C. §103(a) as being unpatentable over the Yi reference in view of the Belmont reference, and further in view of the Earles and Kurano references. With regard to claim 17, the Office Action argues that it would have been obvious to one with ordinary skill in the art at the time of the invention to add oxygen as taught by the Earles and Yi references because

the oxygen will keep the cells or tissues viable and thus increase the signal strength of the electrochemical property. With regard to claims 18 and 19, the Office Action argues that the source of oxygen is implied because the oxygen is a stream of 95% O₂ – 5% CO₂ and that Figure 1 of the Yi reference discloses a carbon fiber electrode positioned adjacent to a substrate (respectively). With regard to claim 21, the Office Action additionally argues that having the resistant region of the Yi reference, as modified by the Earles and Kurano references, sized for attachment of only one cell is just an obvious variant of monitoring a few cells.

As discussed above, amended claim 16 includes the additional feature that a carbon fiber electrode extends at an angle relative to the at least one attachment region. Because the Yi reference does not disclose an electrochemical monitoring system having a carbon fiber electrode extending at an angle to the substrate which, Applicants respectfully submit that amended claim 16 is not obvious in view of the Yi and Belmont references, and further in view of the Earles and Kurano references. Because claims 17-18 and 19 depend either directly or indirectly from claim 16, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claims 17-18 and 19 be withdrawn.

5. 35 U.S.C. §103(a) rejection of claims 7-9 and 13-14.

Claims 7-9 and 13-14 were rejected under 35 U.S.C. §103(a) as being unpatentable over the Yi reference in view of the Earles and Kurano references, and further in view of the Belmont reference. With regard to claim 7, the Office Action argues that it would have been obvious to one with ordinary skill in the art at the time of the invention to create a hydrophobic region on the glass slide which resists

attachment of cells because this will prevent the cells from falling or moving off of the slide. With regard to claim 8, the Office Action argues that the method of the Yi reference as modified by the Earles and Kurano references to monitor only one cell is just an obvious variant of monitoring few cells. Regarding the limitations of claims 9, 13 and 14, the Office Action respectively cites the separated cells in Figure 1, the abstract and first paragraph of the *Introduction*, and the section entitled Measurement of doxorubicin concentrations close to the cell monolayer in the Yi reference.

As discussed above, amended claim 1 includes the further feature that “the step of adding oxygen to the medium including bubbling an oxygen-containing gas into the medium.” The Office Action states that the Danek reference as modified by the Earles reference teaches away from the feature requiring “the step of adding oxygen to the medium including bubbling an oxygen-containing gas into the medium” because “[b]ubbling the 95% O₂ – 5% CO₂ directly into the solution during an experiment is not recommended because it is likely to result in bubbles on the electrode surface which interfere with the detection of the compound under study.” Because amended claim 1 includes this additional feature, it is respectfully submitted that amended claim 1 is not obvious over the Earles and Kurano references, and further in view of the Belmont reference. Because claims 7-9 and 13-14 depend either directly or indirectly from amended claim 1, it is respectfully requested that the 35 U.S.C. §103(a) rejection of claims 7-9 and 13-14 be withdrawn.

6. 35 U.S.C. §103(a) rejection of claims 16, 23, 25-27 and 29.

Claims 16, 23, 25-27, and 29 were rejected under 35 U.S.C. §103(a) as being unpatentable over Parce *et al.* (U.S. Patent No. 5,496,697) (hereinafter, "the Parce reference") in view of the Belmont reference. With regard to claim 16, the Office Action argues that the Parce reference discloses an apparatus for measuring efflux of a chemical from a biological cell, or a population of cells, the apparatus including a substrate having a surface which receives the cell, a medium on the substrate, and an electrochemical monitoring system which measures an electrochemical property of the medium surrounding the cell, the property being related to a concentration of the chemical in the medium. The Office Action also argues that it would have been obvious to one with ordinary skill in the art at the time of the invention to create a hydrophobic region on the substrate which resists attachment of the cells to prevent the cells from falling or moving off of the slide.

With regard to claim 27, the Office Action argues that the Parce reference discloses a method of measuring efflux of a chemical from a biological cell, or a population of cells, the method including introducing the chemical to the cell and measuring a property of a medium surrounding the cell or population of cells, the property being related to a concentration of the chemical in the medium, the method characterized by positioning the cell or population of cells on a surface of a substrate by attachment of the cell or cell population to a region of the substrate which permits attachment. The Office Action also argues that it would have been obvious to one with ordinary skill in the art at the time of the invention to create a hydrophobic region

on the substrate which resists attachment of the cells to prevent the cells from falling or moving off of the slide.

With regard to claim 29, the Office Action argues that the Parce reference discloses a method of measuring transport of a chemical across a membrane of a human or other biological cell, the method comprising exposing the cell to the chemical and measuring a property of a liquid medium disposed outside the cell, the property being related to a concentration of the chemical in the medium, the method characterized by: providing a substrate surface with a region formed from a material to which the cell attaches, the region being surrounded by a portion of the surface which resists attachment of a cell; patterning the substrate using photolithographic techniques to define at least one sensor adjacent the attachment region for sensing the property of the liquid medium; depositing the cell on the region; and after the step of exposing the cell to the chemical, detecting the property of the liquid medium surrounding the cell and determining the concentration of the chemical in the medium therefrom. The Office Action also argues that it would have been obvious to one with ordinary skill in the art at the time of the invention to create a hydrophobic region on the substrate which resists attachment of the cells to prevent the cells from falling or moving off of the slide.

As discussed above, amended claim 16 includes the additional feature that a carbon fiber electrode extends at an angle relative to the at least one attachment region. Neither the Parce reference nor the Belmont reference discloses an electrochemical monitoring system having a carbon fiber electrode extending at an angle to the substrate. Accordingly, Applicants respectfully submit that amended

claim 16 is not obvious in view of the Parce and Belmont references, and respectfully request that the 35 U.S.C. §103(a) rejection of claim 16 be withdrawn. Because claims 23 and 25-26 depend directly from claim 16, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claims 23 and 25-26 also be withdrawn.

It is respectfully submitted that the Parce and Belmont references do not teach or disclose the method of amended claim 27. Amended claim 27 recites the feature that the electrochemical system include a carbon electrode which extends at an angle relative to the at least one attachment region for measuring the electrochemical property of the medium surrounding the cell. Neither the Parce reference nor the Belmont reference discloses a working electrode which extends at an angle relative to an attachment region. Because claim 27 includes the feature that the working electrode include a carbon electrode which extends at an angle relative to the substrate, Applicants respectfully submit that amended claim 27 is not obvious over the Parce and Belmont references. Accordingly, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claim 27 be withdrawn.

As amended, claim 29 recites a method of measuring transport of a chemical across a membrane of a human or other biological cell, the method comprising exposing the cell to the chemical and measuring an electrochemical property of a liquid medium disposed outside the cell, the property being related to a concentration of the chemical in the medium, the method characterized by: providing a substrate surface with a region formed from a material to which the cell attaches, the region being surrounded by a portion of the surface which resists attachment of a cell; patterning the substrate using photolithographic techniques to define at least one

sensor adjacent the attachment region for sensing the property of the liquid medium; depositing the cell on the region; and after the step of exposing the cell to the chemical, detecting the property of the liquid medium surrounding the cell and determining the concentration of the chemical in the medium therefrom, the electrochemical system including a carbon electrode which extends at an angle relative to the at least one attachment region for measuring the electrochemical property of the medium surrounding the cell. Support for amended claim 29 can be found on page 32, line 30, to page 42, line 24, of the present Application.

It is respectfully submitted that the Parce and Belmont references do not teach or disclose the method of amended claim 29. Amended claim 29 recites the feature that the electrochemical system include a carbon electrode which extends at an angle relative to the at least one attachment region for measuring the electrochemical property of the medium surrounding the cell. Neither the Parce reference nor the Belmont reference discloses a working electrode including a carbon electrode which extends at an angle relative to the attractive region of the substrate. Because claim 29 includes the feature that the working electrode include a carbon electrode extending at an angle relative to the at least one attachment region, Applicants respectfully submit that amended claim 29 is not obvious over the Parce and Belmont references. Accordingly, Applicants respectfully request that the 35 U.S.C. §103(a) rejection of claim 29 be withdrawn.

7. 35 U.S.C. §102(e) rejection of claim 30.

Claim 30 was rejected under 35 U.S.C. §102(e) as being anticipated by Farb *et al.* (U.S. Patent No. 6,048,722) (hereinafter, "the Farb reference"). The Office

Action argues that the Farb reference discloses a method of measuring transport of a chemical across a membrane of a biological cell, the method comprising exposing the cell to the chemical, the method characterized by: providing a substrate surface with a site formed from a material to which the cell attaches, the site being surrounded by a portion of the surface which resists attachment of a cell; depositing the cell on the site; moving a sensor through a wall of the cell to contact material in the cell; and measuring a property of a material within the cell with the sensor, the property being related to a concentration of the chemical in the cell and determining the concentration of the chemical in the cell therefrom.

It is respectfully submitted that amended claim 30 is not anticipated by the Farb reference because the Farb reference does not teach every element of amended claim 30. To anticipate a claim, the reference must teach every element of the claim (MPEP 2131). The Farb reference does not teach the step (*i.e.*, element) of claim 30 which recites "providing a substrate surface with a site formed from a material to which the cell attaches, the site being surrounded by a portion of the surface which resists attachment of a cell." Additionally, the Office Action fails to identify this element in the rejection of claim 30. Because the Farb reference does not teach every element of amended claim 30, Applicants respectfully submit that claim 30 is not anticipated by the Farb reference. Accordingly, Applicants respectfully request that the 35 U.S.C. §102(e) rejection of claim 30 be withdrawn.

8. 35 U.S.C. §112, second paragraph, rejection of claims 21, 27 and 30.

Claims 21, 27, and 30 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim

the subject matter which Applicants regard as the invention. The Office Action states that there is insufficient antecedent basis for: the limitation "hydrophilic region" in line 3; the limitation "the attractive region" in claim 27; and the limitation "the material" in claim 30.

Applicants have amended claims 21, 27, and 30 so that the terms "hydrophilic region," "the attractive region," and "the material" each include the proper antecedent basis, respectively. Accordingly, Applicants respectfully request that the 35 U.S.C. §112, second paragraph, rejection of claims 21, 27, and 30 be withdrawn.

9. Objections to claims 1 and 7.

Claims 1 and 7 were objected to because of grammatical informalities. The Office Action states that "cell," in claim 1 should be replaced with "cell", and that "cells" should be replaced with "cells,". The Office Action also states that the word "at" in claim 7 should be deleted.

Applicants have amended claims 1 and 7 as requested in the Office Action and thus respectfully request that the objections to claims 1 and 7 be withdrawn.

10. Patentability of new claim 31.

Claim 31 has been added by the present Amendment and recites an apparatus for measuring efflux of a chemical from a biological cell, or a population of cells, the apparatus including: a substrate having a surface which receives the cell; a medium on the substrate; and an electrochemical monitoring system which measures an electrochemical property of the medium surrounding the cell, the property being related to a concentration of the chemical in the medium. The apparatus is characterized by: the substrate surface having at least one attachment

region to which the cell or population of cells attaches, the region being surrounded by a resistant region which resists the attachment of cells; the electrochemical system including a carbon electrode defining an annulus which surrounds the at least one attachment region. Support for claim 31 can be found at page 14, line 25, to page 15, line 3, of the present Application.

New claim 31 includes the subject matter of claim 20, which was indicated as being allowable if written in independent form by the Office Action. Accordingly, Applicants respectfully submit that that new claim 31 should be allowable by the present Amendment.

In view of the foregoing, it is respectfully submitted that the above-identified application is in condition for allowance, and allowance of the above-identified application is respectfully requested.

Please charge any deficiency or credit any overpayment in the fees for this amendment to our Deposit Account No. 20-0090.

Respectfully submitted,



Richard S. Wesorick
Reg. No. 40,871

TAROLLI, SUNDHEIM, COVELL,
& TUMMINO L.L.P.
1300 E. 9th Street, Suite 1700
Cleveland, Ohio 44114
Phone: (216) 621-2234
Fax: (216) 621-4072
Customer No.: 26,294